Study to investigate the pancreatic regeneration and evaluation of the antidiabetic and antihyperlipidemic potential of aerial parts of *Allium cepa*


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ABSTRACT

The aim of this study was carried out to investigate, clarify the pancreatic regeneration, and recommend in different fractions of the ethanol extract *Allium cepa* (onion) L. Antidiabetic and antihyperlipidemic potentials were reported in streptozotocin – induced diabetic rats and its *in vivo* and *in vitro* antioxidant effects were studied. The extract and their obtained fractions were initially screened for acute and sub-chronic antidiabetic activity on dose with 100 to 200 mg/kg. The main potent extract and fractions were evaluated for pancreatic ß-cells regeneration effect as well as antioxidant and antihyperlipidemic activity. The polyphenolic, flavonoid and flavanone contents were assessed and correlated with its antidiabetic activity. The indicated pancreatic regeneration effect, antidiabetic and antihyperlipidemic activity have been demonstrated by ethanol extract and butanol soluble fraction within a dose level of 200 mg/kg, maintrance rutin was shown to be least less potent. Studies of ethanol extract treated rats showed pancreatic regeneration in the form of nesidioblastosis. The remaining newly generated islets may have been formed from the ductal precursor cells and are able to decrease oxidative stress and helps in restoration of ß-cells function.

Keywords: Antihyperlipidemic, antidiabetic, antioxidant, *Allium cepa*, ß-cells regeneration.

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INTRODUCTION

Diabetes mellitus is a metabolic disorder of carbohydrate, fat, and protein metabolism resulting in hyperglycemia due to defects in insulin secretion, insulin action or both. Oxidative stress is the main factor correlated to the development of glucose toxicity in diabetes. It can even cause lowering in the level of some insulin transcription factors, such as PDX – 1 – and MafA. These proteins usually bind to insulin promoter and stimulate insulin gene transcription, so reduced levels of the two mention proteins cause decrease in insulin promoter activity, insulin gene expression and insulin secretion (Robertson and Harmon, 2006).

Most oral antidiabetic drugs provide only symptomatic relief and are inactive to treat diabetics. On the other hand, a lot of herbs are being used for their potential pancreas regeneration properties, which led to controlled studies on their effectiveness and potential risks. Some of these products allow the regeneration of ß-cells in both
human and non-human subjects. For example, chard extract (Beta vulgaris L. var. cicla) has been used as a hypoglycemic agent by DM patients in Turkey (Bolkent et al., 2000; Chakravarthy et al., 1980, 1981; Subash–Babu et al., 2009). These herbs flavonoids are the most common and are widely distributed group of plant phenolics (Harborne, 1986) consumed (Nakamura et al., 2000).

Chard extract (B. vulgaris L. var. cicla) has been used as a hypoglycemic agent by DM (diabetes mellitus) patients in Turkey (Bolkent et al., 2000). It is regarded as one of the earliest documented extracts with a B - cells regeneration capacity. Whatever, and it was shown that B -cells secretion granules increase after treatment with this plant extract (Nakamura et al., 2000). Same as a many phytoconstituent such as epicatechin, quercetin, rutin, nymphaoyer and flavonol extract obtained from pterocarpus marsupium have also shown to possess B -cells regeneration activity (Chakravarthy et al., 1980, 1981; Limbert et al., 2008; Subash–Babu et al., 2009). Flavanoids represent the most common and widely distributed group of plants phenolics (Harborne, 1986) and are abundant in foods; quercetin and rutin are the flavonoids most abundantly consumed (Nakamura et al., 2000).

A. cepa is a rich, highly effectively legume used as a livestock forage plants in many countries (Gomez and kalaman, 2003; Hall, 1992). It is proven is to be the most widely used herbal medicines in a lot of countries (Gomez and Kalamani, 2003; Hall, 1992). Phytochemical investigations of A. cepa have reported the presence of flavonoid glycosides such as rutin, delphinidin, quercetin, kaempferol and malvidin. The plant is usually used in Ayurvedic medicine, as a nootropic, anxiolytic, antidepressant, tranquilizing and sedative agent. Its extracts possess a wide range of pharmacological properties as anti-inflammatory, antiadibetic, antimicrobial, antipyretic, antilasthmatic, hepatoprotective analgesic, diuretic, local anesthetic, insecticidal, blood aggregation–inhibiting and for use as a vascular smooth muscle relaxing activity (Kalyan et al., 2011; Nithianantham et al., 2013; Taur and Patil, 2011; Mukherjee et al., 2008).

In the present study, aqueous and ethanol extract of roots, leaves and flowers, that is, A. cepa have been investigated for its antiadibetic potential, the mechanism of action and activity of flavonoid rich fraction of the herb on pancreatic regeneration properties was not evaluated keeping the above information in view (Daisy et al., 2009; Mukherjee et al., 2008).

MATERIALS AND METHODS

Animals

Adult male albino rats (175 to 200 g) were obtained from the animal house of a medicinal university, housed at 22 ± 5°C in an air-conditioned room and supplied with standard pellet food with tap water ad libitum. All rats received human care according to the criteria outlined in the guide for care used in the protocols which were approved by the National Academy of Science and published by the National institutes of health.

Preparation of A. cepa aqueous extract and its fractions

Fresh aerial parts of A. cepa, were collected, washed, air-dried under shade and pulverized into a coarse powder. The powder crude material was defatted with petroleum ether and then extracted with ethanol. The ethanol extract (ACAE) was concentrated in a rotary evaporate and resulted in a dark brown mass. The yield from various extraction were pooled together, giving an average yield of 20.78% w/w for fractionation, the ethanol extract was subjected to liquid–liquid partition with chloroform to yield a chloroform soluble fraction of ethanol extract (ACAC; yield: 18.92% w/w) and a chloroform insoluble fraction. The chloroform insoluble fraction was further fractionated with n–butanol to yield a n–butanol soluble fraction of ethanol extract (ACABS; yield: 55.83% w/w) and a n–butanol insoluble fraction (ACABIS; yield: 25.25% w/w). And ACABIS were subjected to phytochemical and pharmaceutical screening (Stahl, 1969; Stanojevic et al., 2009).

Phytochemical screening

The ACAE extract and its broad fractions were screened for the presence of saponins, tannins, alkaloids, triterpenes, unsaturated sterols, flavonoids, lactones/esters, protein/amino acids, carbohydrates and/or glycosides, using thin layer chromatography (TLC) (Stahl, 1969). Thin layer plates precoated with silica gel G (Merck, 0.25 mm thickness) were used. Development was carried out with different solvent system such as ethyl acetate : methanol : water (100 : 13.5 : 10, v/v/v), ethyl acetate formic acid : acetic acid : water (100 : 11 : 26, v/v/v), toluene : methanol (80 : 20, v/v), toluene : ethyl acetate : formic acid (78 : 18 : 4, v/v/v) and ethyl alcohol : (ACAc) : acetic acid : ethyl alcohol : hydroxylamine : ferric chloride : solution, ninhydrin and antimony trichloride for the detection of alkaloids solution, ninhydrin and antimony trichloride for the detection of alkaloids, flavonoids, lactones/esters, protein/amino acids, unsaturated sterols and triterpenes respectively. While detection of anthraquinones, saponins, tannins, carbohydrate and/or glycosides used KOH, anisaldehyde-sulphuric acid reagent, ferric chloride and naphthoresorcinol reagent respectively. Visualization was measured under visible light (λ 366 nm). ACAE extract and its fractions were also quantified for presence of important secondary metabolites such total polyphenol, flavonoid and flavonone compounds, using the following spectroscopic methods:

Study of total polyphenol (TP), flavonoids (TFA) and total flavanones (TFO)

Total polyphenol was determined by the Folin–Ciocalteu colorimetric method (Singleton et al., 1999). Gallic acid was used as a reference for standard curve construction. The results were mention in mg of gallic acid equivalents (GAE)/g of extract. Flavonoid content was studied by the aluminum chloride method (Stanojjevic et al., 2009). Rutin was used as a reference for standard curve construction. For studied of flavonones the modified 2, 4- dinitrophenylhydrazine method may be used (Nagy and Grancai, 1996). Naringin was used as a reference standard and results were obtained in mg of naringin equivalents NE/g of extract. All results were carried out in triplicate (Chang et al., 2002).

Quantitative evaluation of rutin by HPLC (Gupta et al., 2006)

Crude extracts and its fractions were standardized and also
estimated in relation to their rutin content by HPLC. 10 µl of samples test and standard solution were used on a precoated silica gel G 60 F-254 TLC plate (E. Merck). Chromatogram was applied ethyl acetate: formic acid: ethanol: water: (100: 11: 11: 26) (Marston and Hostettman, 2006), measured at 366 nm and studied against rutin standard for quantification with area under the curve method.

**Assay of in vitro antioxidant studies**

The antioxidant activities of rutin, ethanol extract and its fractions were assessed by 2, 2-diphenyl - 1 - picrylhydrazyl (DPPH) method (Sharma and Bhat, 2009) and nitric oxide (NO) radical scavenging method (Tsikas, 2007). Ascorbic acid was used as a reference standard and all results were expressed in triplicates.

**Acute toxicity studies**

Rats were randomly divided into test and control groups (n = 6). The test group was administered oral dose (1, 3 and 5 g/kg) of extract and its fractions. In all instances, food and water ad libitum and were kept under suitable condition and regular observation for symptoms of mortality and behavioural changes for a 48 h period (Dixon, 1965; OECD, 2000).

**Experimental procedure**

The induction of diabetes was induced by sub – cutaneous (s.c.) injection of streptozotocin (STZ) in a single dose of 45 mg/kg body weight. STZ was prepared by dissolved in 0.01 M citrate buffer (pH 4.5). Rats were allowed to take 5% glucose solution overnight which caused the drug induced hypoglycemia. Twenty four hours after the injection, blood was withdrawn from overnight fasting animals and blood glucose level was assessed by a glucometer. The fasted rats (8 h) with blood glucose level reached 200 mg/dl were selected for the experiment as diabetic rats. Control group were given normal saline alone (Banskota et al., 2006).

**Experimental design**

**Acute antidiabetic studies**

Following induction of diabetes, the animals were divided randomly into eleven groups (1 × 1) of six rats each (n = 6) as follows:

1. Group I, received vehicle (distilled water 10 ml/kg, orally).
2. Group II, administrated insulin (4 IU/kg, s.c.).
3. Group III, received rutin (100 mg/kg, p.o.).
4. Group IV to XI, received ACAE extract and its fractions in a dose range of 100 after administration by oral route using syringe, antidiabetic effect was assessed by withdrawing blood samples within 0, 1, 3, 5, and 24 h respectively. Results were given in mg/dl (Itankar et al., 2011).

**Sub–chronic antidiabetic studies**

The animals were divided into six groups of six rats each (n = 6).

1. Group I; served as diabetic untreated control (fed standard and water only).
2. Group II; served as standard and was treated with 4 IU/kg/day insulin.
3. Group III; received rutin (100 mg/kg p.o.)
4. Group IV to VI received 200 mg/kg of ACAE extract; ACACS fraction and ACABS fraction, respectively orally per day for 21 days.

Fasting blood glucose levels (8 h fasting) and body weight, were measured on day 0, 7, 14 and 21 of the study (Salahuddin and Jalaipure, 2010). Lastly on day 21, blood was collected from retro-orbital plexus, the rats were sacrificed, pancreas was removed, cleaned and washed in ice–cold normal saline for biochemical and histopathological study.

**Pancreatic regeneration studies**

**Tissue processing**

After rats were sacrificed, pancreas were removed, cleared of fat and lymph nodes, weighed and immersed within 1 h in 10% formalin and dehydrated by alcohol. The tissue was embedded in paraffin. Each gland was sectioned serially (4 µm in thickness) on a rotary microtome (Shandon, S325, England) along its length to avoid any bias due to change in islet distribution or cell composition. For each pancreas, ten sections were randomly chosen at a fixed interval through the block (every 35th section), a pancreas was shown to indicate the selected sections that are representative of the whole pancreas (Movassat et al., 1997), and then stained with haematoxylin and eosin (H and E) for histological analysis (Ahmed et al., 2010).

**Histopathological and morphometric test**

The morphological analysis consist on the main area (µm²) of individual islets, perimeter of individual islet as a determination of islet size, and the number of islets per square millimeter was determined on H and E-stained sections of each group on a Motic digital microscope equipped with Motic image plus 2.0 software (Motic, China). To determine the cross-sectional area of focal tissue, sections were systematically sampled using an image analysis system. Slides were tested in 1.0 × 1.5 mm increments, resulting in the assessment of 70 ± 5 fields per side and 82 ± 5% of total pancreatic section.

Individual islets were quantified by capturing each field and, computer assisted, hand tracing focal area within the captured images. The thresholding option was used to quantify the cross sectional areas occupied by tissue and subtract unsummarized, unoccupied areas (white space). The magnification was calibrated using a calibration grid micrograph. Apoptotic cells were photographed from these samples. To evaluate the distribution of islet size of perimeter < 500 µm were classified arbitrarily as small, medium (perimeter between 500 and 1000 µm), and extra-large (perimeter between 2000 and 2500 µm).

**Determination of biochemical parameters**

Antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH) and lipid peroxidase (LPO) were assessed in erythrocyte and pancreas methods used by Jamali and Smith (1985). Serum triacylglycerides (TG), total cholesterol (TC) and high density lipoprotein (HDL) average was also determined (Muller et al., 1977) using standard kits extracted from Span Diagnostic England. Serum low-density lipoproteins (LDL) and very low–density lipoproteins (VLDL) levels were calculated by using the following formula:

\[
\text{VLDL} = \text{TG}/5 \\
\text{LDL} = \text{TC} - (\text{HDL} + \text{VLDL})
\]

**Acute toxicity studies**

Animals were divided into test and control groups (n = 5). The test
group was administrated an increasing oral dose (1, 3 and 5 g/kg) of TPLE (Tephrosia purpurea Linn extract) and its fractions. The animals were supplied food with tap water ad libitum and were kept under regular condition for symptoms of mortality and behavioral changes over a period of 48 h (Dixon, 1965; OECD, 2000).

**Statistical analysis**

The results concerning acute and sub–chronic anti diabetic study, in vivo antioxidant activity, antihyperlipidemic effects, body weight, and morphometric study were expressed as mean ± SD. Statistical differences between the treatments and the controls were examine by two–way analysis of variance (ANOVA) followed by Bonferroni post–analysis using the “GraphPad – Prism” (version 5.02) statistical analysis software. A difference in the main values of p < 0.05 or less was considered statistically significant.

**RESULTS**

**Phytochemical screening**

The phytochemical screening ACAE extract showed the presence of alkaloids, phenolic compounds, tannins, flavonoids, carbohydrates, sterols, terpenoids and proteins. ACABS fraction proved presence of tannins and flavonoids, and ACABIS fraction contained, carbohydrates, proteins, flavonoids, and tannins.

**Acute toxicity studies**

In the test analysis, there was no mortality observed at doses up to 2 g/kg (p. o.) in animals. While during the observation, the animals exhibited decreased mobility but no symptoms of convulsions or loss of writhing reflex. The study determined that ACA extract and its broad fraction indicate a low toxicity profile.

**Study of total polyphenol, flavonoid and total flavanones**

The total polyphenol content (mg/g) can be determined by Foin – Ciocalteu colorimetric method results as 24.24 ± 0.01, 6.51 ± 0.01, 48.06 ± 0.05 and 5.77 ± 0.01 (GAE mg/g of extract) for ACAE, ACACS and ACABS, respectively. Polyphenol content was studied by a linear regression equation of gallic acid and expressed as GAE of extract (y = 0.009x + 0.028, r² = 0.996). The flavonoid content obtained by aluminium chloride methods was found to be 3.28 ± 0.02, 2.9 ± 0.01, 8.89 ± 0.41 and 2.64 ± 0.02 RE mg/g of extracts for ACAE, ACACS, ACABS, and ACABIS respectively. Flavonoid content was obtained from linear regression equation of rutin (y = 0.014x – 0.017, r² = 0.996). The flavanone content obtained by 2.4-dinitrophenylhydrazine method was found to be 1.12 ± 0.02, 0.186 ± 0.01, and 2.86 ± 0.06 and 0.09 NE mg/g of extract for ACAE, ACACS, ACABS and ACABIS respectively. Flavanone content was obtained from linear regression equation of naringin (y = 0.156x + 0.564, r² = 0.976).

The suggestion of Chang et al. (2002) indicated that flavonoids, flavonol and isoflavones formed complexes only with aluminium chloride, in the other hand flavanone strongly reacted only with 2,4-dinitrophenylhydrazine, while the content obtained by the two methods were added up result in total flavonoid content. The content of flavanoid and flavanone represent only 13.53% (w/w) and 4.62% (w/w) of the TP (total polyphenol) in ACAE extract respectively same as pattern was showed in all its fractions, suggesting that the extracts are very complex and having various other polyphenols such as flavanones, isoflavones, phenolic acids and tannins, and the degree of polymerization of the polyphenols shown in the samples is high. Degree of polymerization can be determined by the ratio between TP and TFA (total flavonoids) contents (Souza et al., 2008). The highest polymerization is showed in ACAE fraction and it varies from 7.39, 2.9, 5.41 and 2.18 for ACAE, ACACS, ACABS and ACABIS respectively. The fractionation of ethanol extracts ACAE within a different solvent obtained is a suggestion of important secondary metabolites such as steroids. Moderately hydrophobic polyphenols were isolated in ACACS fraction, so flavonoids and majority of polyphenols were enriched in ACABS fraction.

**Quantitative study of rutin**

Rutin extract content and its fraction were found to be 0.15, 0.32 and 0.08% w/w in ACAE, ACABS and ACABIS respectively. Whatever; these investigation demonstrate that fraction of the extract resulted in n–butanol fraction.

**In vitro antioxidant studies**

To investigate the mechanism of antioxidant capacity, various different analytical methods varying in their oxidation initiators and targets were used. Antioxidant capacity of an extract shown depends on its composition and the methodology used. This is the same as the oxidant and the oxidisable substrate used in the measurement (Cao and Prior, 1998). In other side, the characteristic of a particular test reaction can influence the analysis outcome, so others are insensitive in this matter.

The antioxidant result related of the rutin extract and its fractions were showed in lowering order of rutin > ACABS > ACACS > ACAE > ACABIS having IC50 (µg/ml) value of 1.26 ± 0.01, 605 ± 0.02, 21.95 ± 0.04, 36.79 ± 0.03, 67.14 ± 0.01 and 19.79 ± 1.01, 169.63 ± 1.09, 182.25 ± 3.98, 254.47 ± 2.01, 324.52 ± 6.25 in DPPH and NO scavenging method, respectively.
Table 1. Effect of Allium cepa aerial parts extract and its fraction in acute antidiabetic study.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>553.75 ± 17.97</td>
</tr>
<tr>
<td>Insulin 4 IU/kg, p.o.</td>
<td>573.3 ± 9.12</td>
</tr>
<tr>
<td>Rutin (100 mg/kg,p.o.)</td>
<td>528 ± 10.61</td>
</tr>
<tr>
<td>ACAE (200 mg/kg,p.o.)</td>
<td>541.30 ± 14.26</td>
</tr>
<tr>
<td>ACACS (200 mg/kg,p.o.)</td>
<td>533.26 ± 29</td>
</tr>
<tr>
<td>ACABS (100 mg/kg,p.o.)</td>
<td>524 ± 8.40</td>
</tr>
<tr>
<td>ACABS (200 mg/kg,p.o.)</td>
<td>524 ± 6.38</td>
</tr>
<tr>
<td>ACABIS (100 mg/kg,p.o.)</td>
<td>531.84 ± 1078</td>
</tr>
<tr>
<td>ACABIS (200 mg/kg,p.o.)</td>
<td>540 ± 10.35</td>
</tr>
</tbody>
</table>

ACAE, aerial part ethanol extract; ACACS, chloroform soluble fraction of ethanol extract. CAABS, n-butanol soluble fraction of ethanol extract.

a: The data are expressed in mean ± S.D n = 6 in each group.
b: Represent statistical significance vs Diabetic control (p < 0.01).
c: Represents statistical significance vs Diabetic control (p < 0.001).

Antihyperglycemic effect

Acute toxicity studies explained the non-toxic nature of ACAE extract and its fractions. There was no toxic effect observed up to a dose level of 2 g/kg, p. o. The ACAE extract, its fractions, and rutin, have been showed to induce a significant effect in blood glucose levels in an acute antidiabetic study (Table 1). The insulin (short-acting soluble human insulin) at dose level of 4 IU/kg indicated 80.98% reduction in plasma glucose after 24 h (574.3 ± 9.11 mg/dl at 0 h to 109.43 ± 8.52 mg/dl; p < 0.001). Amongst the extract and its fraction ACAE extract has decreased highest blood glucose level to 66.0% dose level at 200 mg/kg P.O. after 24 h (541.30 ± 14.26 mg/dl at 0 h to 184.0 ± 7.87 mg/dl; P < 0.001), also comparing with blood glucose at 0 h. Oral administration of rutin also significantly reduced 50.19% blood glucose level after 24 h (528 ± 10.61 mg/dl at 0 h to 263 ± 6.78 mg/dl; p < 0.001). The 66.0% reduction in blood glucose level exhibited by ACAE extract 200 mg/kg P.O. at 24 h is similar to that distributed into chloroform (ACACS), and n-butanol (ACABS) soluble fractions in 55.23 and 46.14% respectively; while ACABIS fraction did not indicate any antidiabetic effect in both the selected doses. However, none of the extract fractions was as a potent antidiabetic as insulin. Thus, it is evident that the above study that determined a higher antidiabetic potential of ACAE extract than its fraction may be such as of synergistic activity of constituent’s presents in it, which was distributed in its fraction.

In a sub-chronic antidiabetic observation, it was shown that prior to the extract administration there were no significant differences between the fasting blood glucose levels of the diabetic groups of animals. However, after three weeks, the fasting blood glucose levels of the treated animals were significantly reduced than the diabetic controls. In others studies the blood glucose level of the untreated diabetic animal remained highest throughout the investigation period. The most prominent antidiabetic studies was shown at ACAE and ACABS fractions (66.02 and 59.32% in comparison with its blood glucose level at day zero) with in a dose level of 200 mg/kg at day 21 of treatment (Table 2). Rutin also reduced the fasting (8 h) blood glucose level to 52.34% compared to diabetic animals at day zero. Studs obtained from this investigation indicate that, ACAE extract and its fractions show antidiabetic studies in reducing order of ACAE > ACABS > rutin > ACACS.

Histological study, morphometric analysis of islet

In STZ – diabetic rats, the islet is considerably reduced and shrunken. There is destruction of some β –cells with central hyalinization; a few cells showed pyknotic nuclei and total cell number reduced (Figure 1). The degree of atrophy, hydropic degeneration, necrosis, hyalinization, or fibrosis was checked in H 7 & E stained sections. Many of the islets were degranulated and high infiltration of T-cell was observed on treatment with STZ (Figure 1B). The average number of islet and average islet area per square of pancreatic samples in µm were determined by computer assisted calculated morphometric studies has confirmed that treatment showed in pancreatic regeneration. The size and distribution pattern explained that islets of normal group animals were larger in size (2000 to 2500 µm perimeter; n = 6). Also the larger islet subsets were also evident in diabetic control group nevertheless, these are mostly degranulated and having central hyalinization with T-cell infiltration (Figure 1A and B). Same as the average number of islets (0.87 ± 0.02 × 10^4 vs. 5.1 ± 0.16 × 10^4 in the control group) was 82.98% less abundant and islet density (2.89 ± 0.09 vs.
Table 2. Effect of *Allium cepa* aerial parts extract and its selected fractions on islet.

<table>
<thead>
<tr>
<th>Distribution of islet size (µm; perimeter)</th>
<th>Normal control</th>
<th>Diabetic control</th>
<th>Insulin (IU/kg s.c.)</th>
<th>Rutin (100 mg/kg, p.o.)</th>
<th>ACAE (200 mg/kg; p.o.)</th>
<th>ACACS (200mg/kg; p.o.)</th>
<th>Distribution of islet as per size</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;500</td>
<td>2 ± 0.15</td>
<td>0</td>
<td>6 ± 0.15</td>
<td>16.6 ± 0.23</td>
<td>17.2 ± 1.28</td>
<td>10 ± 1.2a</td>
<td>12.46±0.57</td>
</tr>
<tr>
<td>500 - 1000</td>
<td>2 ± 0.3</td>
<td>0</td>
<td>4 ± 0.12</td>
<td>3 ± 0.18b</td>
<td>4 ± 0.13b</td>
<td>1 ± 0.17</td>
<td>2±0.24</td>
</tr>
<tr>
<td>1000 - 1500</td>
<td>4 ± 0.1</td>
<td>1 ± 0.21</td>
<td>2 ± 0.6</td>
<td>2 ± 0.52</td>
<td>3 ± 0.05</td>
<td>1 ± 0.72</td>
<td>1.18±0.85</td>
</tr>
<tr>
<td>1500 - 2000</td>
<td>4 ± 0.01</td>
<td>4 ± 0.08</td>
<td>0</td>
<td>1 ± 0.02</td>
<td>1 ± 0.02b</td>
<td>0</td>
<td>2±0.2</td>
</tr>
<tr>
<td>2000 - 2500</td>
<td>5 ± 0.21</td>
<td>4 ± 0.01</td>
<td>2 ± 0.11</td>
<td>0.34 ± 0.01</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Average no. of sq. µm of pancreas (&gt;10³)</td>
<td>051 ± 0.016a</td>
<td>0.868 ± 0.0021</td>
<td>0.28 ± 0.002</td>
<td>0.41 ± 0.08a</td>
<td>0.712 ± 0.022b</td>
<td>0.43 ± 0.001b</td>
<td>0.625 ± 0.085b</td>
</tr>
<tr>
<td>Average size of islet (µm×10⁻⁵)</td>
<td>769.9 ± 12.9a</td>
<td>1167.8 ± 23.7c</td>
<td>845.4 ± 49.8b</td>
<td>328.5 ± 15.2a</td>
<td>286.8 ± 15.3a</td>
<td>162.4 ± 18.5a</td>
<td>239.2 ± 12.6a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Distribution of islet size (µm; perimeter)</th>
<th>Normal control</th>
<th>Diabetic control</th>
<th>Insulin (4IU/kg; s.c.)</th>
<th>Treatment</th>
<th>Rutin (100 mg/kg; p.o.)</th>
<th>ACAE (100 mg/kg; p.o.)</th>
<th>ACACS (1200mg/kg; p.o.)</th>
<th>Distribution of islet as per size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average islet area per sq. µm of pancreas</td>
<td>3.285 ± 0.24a</td>
<td>0.288 ± 0.16b</td>
<td>0.501 ± 0.012</td>
<td>0692 ± 0.09a</td>
<td>09235 ± 0.03a</td>
<td>0.512 ± 0.09a</td>
<td>0.806 ± 0.003a</td>
<td></td>
</tr>
</tbody>
</table>

ACAE, ethanol extract; ACACS, chloroform soluble fraction of ethanol extract; ACABS, n-butanol soluble fraction of ethanol extract.
The data are expressed in mean ± S. D N = 6 in each group.
a: P < 0.05.
b: P < 0.01.
c: P < 0.001.

3.29 ± 0.24 per sq. µm of pancreas in normal animals) was 91.23% less in per sq. µm in pancreas compared with normal rats. Furthermore, average area of islet increased 1.52 fold (769.9 ± 12.9 vs. 1167.8 ± 23.7 in diabetic control; p < 0.05) in diabetic animals than normal animals. Whereas insulin and rutin administration for 21 days also resulted in the increase of islet less than 500 µm in size indicative of islet regeneration. By comparing with the diabetic group, the average number of islet per sq. µm of pancreas increased by 3.22 and 4.72 fold in the insulin and rutin groups, respectively. So compared to the diabetic control, islet density improved by 93.95% in insulin group (5.01 ± 016 × 10⁴ vs. 2.8 ± 0.02 × 10⁴ in diabetic controls) (Figure 1A to D). Thus, from the previous study (sub-chronic antidiabetic study) and morphometric study it was evident that rutin possesses pancreatic regeneration potential and these results are in agreement with previous studies (Guz et al., 2001).

Treatment with ACAE fraction and ACABS extract showed in improvement in β – cell granulation and decreased necrosis, hyalinization, or fibrosis of islets compared to diabetic control animals. However, treatment with ACACS fraction for 21 days indicated in partial improvement in pancreatic tissue integrity. The number of T-infiltrated and degranulated islet cell were decreased in ACACS fraction in compared with diabetic control group. Morphometric analysis indicated that treatment has showed in pancreatic regeneration. The size distribution pattern has revealed the presence of higher number of smaller islets (< 500 µm in perimeter) in ACAE (17.2 ± 1.29; p <0.001 resp.) compared to rutin treated, normal and diabetic control group, thus showed the formation of neo-islet. Such as, ACAE and ACABS fractions resulted in the increase of islet numbers, however, it is less pronounced than in the rutin treated group.

The highest number of islet elevated in ACAE and ACABS by 8.20 and 7.20 fold (0.87±0.02 vs. 7.12 ± 0.22 and 6.25 ± 0.25) in ACAE and ACABS group, compared to the diabetic control group, and these effects were more indicated than insulin and rutin treated groups. Similarly a 320.66% and 279.86% increase in the average islet area per sq.µm was shown in ACAE and ACABS treated groups compared to diabetic control after
treatment for 21 days. However, the increase in islet area of ACAE and ACABS treated groups were 38.55 and 31.06% of islet area of normal control group. Treatment with ACACS fraction had a very marginal effect in the islet size area and distribution pattern in relation to the insulin group, but it was less effective than rutin treated group. The pancreatic regeneration potential showed by rutin treatment was less indicated than that of the ACAE fraction (Table 2).

**In vivo antioxidant studies in erythrocytes and pancreas**

The *in vivo* antioxidant enzyme effects in the erythrocytes and pancreas of normal and diabetic group were assessed. In general, normal groups contained higher enzyme level of SOD, CAT, GSH and lower LPO level than diabetic groups (Sarkhail et al., 2007). Daily administration of extract and its fractions were effective in completely recovering the enzyme effects, as level showed in normal group animals. Treatment with rutin, ACAE, ACACS and insulin significantly elevated level of SOD, CAT and GSH and decreased elevated LPO level to near normal erythrocytes and pancreas in diabetic groups (Table 3).

However, the extract, or fractions showing antidiabetic potential by lowering the production of ROS by inhibiting autoxidation of glucose elicit *in vivo* antioxidant potential.
Study the effect on body weight and lipid profile

There was no significant intra-group change in the basal body weight of the rats at day zero. Also at the end of 21 days of treatment the body weight of the rats in the normal group, insulin group, rutin, ACAE, ACACS and ACABS treated group, elevated significantly in 22.5, 18.83, 16.11, 17.83, 11.17 and 16.41% respectively; while the body weight of diabetic control group reduced in about 22.5%.

In the present investigation, diabetic animals treated with rutin, ACAE extract and its fractions resulted in an increase in body weight as shown in the diabetic control; this may be due to its protective effect in controlling muscle wasting (reversal of gluconeogenesis). The most potent reduction in highest TC, TG, and LDL was showed within insulin group and ACAE extract. The treatment with ACAE extract for 21 days showed 50.28, 72.78 and 64.55% decreased in plasma LDL, TG and total cholesterol and also, 215.90% increase in HDL level in relation to diabetic control. Similarly TC: HDL ratio and LDL: HDL ratio were significantly investigated by 76.76 and 83.27% in ACAE treated group, as compared to diabetic control. Treatment with ACACS and ACABS fraction results in a moderate decrease of elevated TC, TG and LDL percent, while rutin a naturally occurring bioflavonoid results in a lower efficacy in management of lipid profile in relation to selected fractions of A. cepa and its extracts (Table 3).

**DISCUSSION**

The most potent antioxidant effect was determined by rutin in all assays

The higher antioxidant potency of extract and its fractions in DPPH (Sharma and Bhat, 2009 ) assay in relation to the NO assay can be investigated as both DPPH and NO assays, explained the free radical-scavenging effect but DPPH is carried out in an organic environment (an alcohol for example); so NO assay requires a buffered aqueous solution. Also the NO assay is investigated in an aqueous buffer, the nitric oxide molecule itself is a lipophilic species, hence it has a higher compatibility to those components that has been dispersed well in the buffer and interact with the free radical. While the extract and its fractions, ACABS fraction showed the highest antioxidant potential in both assays. These results are in agreement with its chemical content, as fractionation of extract resulted in enrichment of polyphenol and flavonoid components in the ACABS fraction, which as a resolute showed a higher antioxidant effect than ACAE extract. Finally these studies suggested that there could be a correlation between the higher content of total polyphenol, flavonoid contents and its antioxidant (stanojevic et al., 2009).

Antidiabetic investigation reveals that ACAE extract and its fractions have sub-chronic antidiabetic effect in lowering order ACAE > ACACS > ACABS > Rutin. The elevated potential of ACACS fraction over ACABS extract indicate a higher amount of polyphenol and flavonoids, and suggests that fractionation increases the degree of polymerization and segregation of secondary metabolites such as steroid, terpenoids and complex polyphenols, which showed in elevated antidiabetic potential. In contrast to all the fraction and extract in sub-chronic antidiabetic investigate, the decrease antidiabetic potential of well-known bioflavonoid, rutin, may show synergistic activity with different secondary metabolites present in the fraction and extract.

Previous studies have shown that there is a similar relationship between the increase of free radicals, blood glucose and lipid peroxidation (LPO) in diabetes progression (Reddy et al., 2005). Increased LPO impairs membrane function by lowering membrane fluidity and changing the effect of membrane-bound enzymes and receptors.

Antioxidants enzymes such as SOD and CAT are considered as primary enzymes, and they are related to the direct elimination of reactive oxygen species (ROS) (Arulselvan and Subramanian, 2007). SOD is an

Table 3. Effect of aerial parts and leaves of A. cepa extract and their fractions on lipid profile of diabetic rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TC</th>
<th>TG</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>76.1 ± 3.51</td>
<td>35.94 ± 3.24</td>
<td>27.27 ± 4.72</td>
<td>41.65 ± 2.04</td>
<td>7.19 ± 0.65</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>155.33 ± 2.40</td>
<td>156.67 ± 1.67</td>
<td>13.71 ± 0.38</td>
<td>110.29 ± 2.97</td>
<td>31.33 ± 0.33</td>
</tr>
<tr>
<td>Insulin (4 IU/kg,s.c.)</td>
<td>74.2 ± 2.62c</td>
<td>43.48 ± 1.38c</td>
<td>27.05 ± 0.25c</td>
<td>38.45 ± 2.61c</td>
<td>8.70 ± 0.28c</td>
</tr>
<tr>
<td>Rutin (100 mg/kg,p.o.)</td>
<td>84.25 ± 2.66c</td>
<td>60.76 ± 0.28c</td>
<td>19.03 ± 1.03</td>
<td>53.07 ± 3.05c</td>
<td>12.15 ± 0.06c</td>
</tr>
<tr>
<td>ACAE (200 mg/kg,p.o.)</td>
<td>77.23 ± 1.43c</td>
<td>42.65 ± 1.33c</td>
<td>29.6 ± 2.43c</td>
<td>39.10 ± 1.90c</td>
<td>8.53 ± 0.27c</td>
</tr>
<tr>
<td>ACACS (200 mg/kg,p.o.)</td>
<td>81.49 ± 0.64c</td>
<td>57.17 ± 2.50c</td>
<td>20.32 ± 0.53</td>
<td>49.74 ± 1.56c</td>
<td>11.43 ± 0.50c</td>
</tr>
<tr>
<td>ACABS (200 mg/kg,p.o.)</td>
<td>79.27 ± 2.67c</td>
<td>55.93 ± 3.68c</td>
<td>20.82 ± 1.9a</td>
<td>47.27 ± 3.70c</td>
<td>11.18 ± 0.74c</td>
</tr>
</tbody>
</table>

The data are expressed in mean ± S. E. M. n = 6 each group.

a: Represents statistical significance vs. diabetic control (p < 0.05).
b: Represents statistical significance vs. diabetic control (P < 0.01).
c: Represents statistical significance vs. diabetic control (p < 0.01).
essential defense enzyme, it scavenges O$_2^-$ anions from 
H$_2$O$_2$ and then decreases the toxic effects of this and 
other free radical derived from secondary reacting. CAT 
is a hemoprotein, which catalyzes the reduction of 
hydrogen peroxides and is also influenced in the 
detoxification of H$_2$O$_2$ concentrations in vivo (Verma et 
al., 2013).

Hence, in the current study, the effect of SOD and GSH 
decreased in diabetic group as reported before (Gokce 
and Haznedarroglu, 2008) which has been due to the 
enhancement in the excretion of ROS generated by STZ. 
Treatment of diabetics with the extract and fractions 
related to the activity of these enzymatic antioxidants 
might result in decreased oxidative stress as evidenced 
by decrease of LPO. Thus ending the suggestion that 
AACE induces in vivo antioxidants effect by attenuation 
the lipid caused by different forms of free radicals; in 
this way AACE may affect lipid profile in diabetes. 
Furthermore, the in vivo antioxidant study of the rutin, 
exttract and its fractions were showed in lowering order of 
AACE > ACACS > ACABS > rutin. The in vivo antioxidant 
pattern observed is inverted from its in vivo antioxidant 
pattern. This can be explained as the elevated blood 
glucose level in diabetes, not only generates ROS but 
also attenuates the antioxidative mechanisms by 
scavenging enzymes and antioxidant substances. 
Glucose toxicity produces fatty acid metabolism, such as 
auto-oxidation of glyceraldehyde, which generates 
hydrogen peroxide and a ketoaldehydes, and can result 
in a chronic oxidative damage (Shahat et al., 2004). The 
decrease in body weight associated to diabetes mellitus 
has been attributed to the gluconeogenesis result in 
increased muscle wasting and loss of tissue protein 
(Shirwaikar et al., 2005). Hence, the ability of AACE 
extrant and its fractions effectively to control the increase 
in blood glucose levels in diabetic group of animals and 
significant increase in the body weight may be related to 
its antihyperglycemic effects.

The most commonly showed in lipid abnormalities in 
diabetes are hypertriglyceridemia and hypercholesterolemia. A marked increase in the level in 
total cholesterol and a decrease in HDL has been 
shown in diabetic control animals. Insulin deficiency 
results in failure of activation of lipoprotein lipase thereby 
resulting in hypertriglyceridemia, so in diabetes LDL and 
VLDL carry cholesterol to the peripheral tissues to the 
site where they deposited, while HDL transports 
cholesterol from peripheral tissues to the liver and then 
aids its elimination. Thus the highest level in LDL and 
VLDL is as atherogenic.

Furthermore, the ability of AACE extract and its 
fractions to effectively control the elevation in blood 
glucose in diabetic group and significantly increase the 
body weight may be related to their antihyperglycemic 
effect. So from the above studies, it was shown that the 
efficiency of lipid level reductions is attributed to their 
potential to treat hyperglycaemia. This investigation is 
further supported by histopathological studies of AACE 
extract treated rats; there is evidence of nesidioblastosis, 
such as can be observed by the intact ductal connection 
between the islet and connected pancreatic ducts in the 
pancreas of AACE-treated animals (Figure 1F). This 
result is investigations are in agreement with previous 
studies of ductal cells (Kodama et al., 2003).

The notion that required for β cells from a precursor 
pool may remain active in adulthood is enhancement by 
the studies that was done in the adult rats pancreas 
where some islets were found still closely associated to 
ducts. However, it was significantly demonstrated that β-
cell de novo formation from ductal cell is inducible 
(Shinichi et al., 2015). It is evident that the regenerating 
pancreas has the potential to induce islet neogenesis in 
the STZ-induced diabetic animals (Mirela et al., 2012). 
These newly formed islets were then seen to grow into 
larger mature islets, mainly by islet β-cell replication. 
The increase in islets size both in insulin and diabetic control 
group was observed as a result of continuously 
increasing active demand to which the residual β-cells 
are released and in order to compensate for their 
decreased cell number (Wang et al., 1996). Rutin, by its 
ability to scavenge free radical and to inhibit peroxidation, 
prevents streptozotocin induced oxidative stress and 
protects β-cells showing in increased insulin release and 
lowered blood glucose levels. In this study, investigations 
by Vessal et al. (2003) have been observed that 
quercetin, the form of rutin, lowered blood glucose 
concentration and increase insulin secretion in 
have also described that, in streptozotocin-induced 
diabetic rats, quercetin protected pancreatic β-cells 
integrity by decreasing oxidative stress. The elevated 
insulin levels could be also due to the stimulatory activity 
of rutin agent potentiating the stimulating β-cells of the 
islet of Langerhans in diabetic rats (Hii and Howell, 1985) 
observed an increased number of pancreatic islets in 
quercetin treated animals. It can be explained that AACE 
exttract possess the most potent antidiabetic and 
significant in-vivo antioxidant effect by virtue of its 
capacity to induce pancreatic regeneration. The 
approximate normoglycemia caused by the AACE extract 
is either related to the formation of neo-islets rich in β-
cells granulation or by the replication of the existing islets. 
These neo-islets formed by AACE stimulation showed in 
a lowering of fasting blood glucose level by increased 
level of insulin released, which also inhibits atrophy by 
opposing to ubiquitin-proteasome pathway (Verma et 
al., 2013). This may also help in pancreatic regeneration, 
reduction in oxidative stress, decrease in diabetes 
caused elevated lipid levels and reduce level of SOD, 
GSH and CAT enzyme effect.

Generally, it is evident from the study that the aim was 
to clarify that A. cepa exhibited significant pancreatic 
regeneration effect in STZ-induced diabetic animals. 
AACE extract and its fractions proved relevant as
beneficial for improvement in parameters such as body weight and lipid profile, and as a result, they may be valuable in the treatment of diabetes.

REFERENCES


Tsikas D, 2007. Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: Appraisal of the Griess reaction


